IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

Maertens et al Atty. Ref.: **2752-56**

Continuation of Serial No. **08/928,017** Group:

Filed: **October 10, 2001** Examiner:

For: **Purified Hepatitis C Virus Envelope Proteins for Diagnostic and Therapeutic Use**

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October 10, 2001

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Preliminarily amend the application as follows.

IN THE SPECIFICATION

Insert the attached Sequence Listing after the claims pages.

Page 36, delete the paragraph spanning lines 23-29, and insert the following therefor:

--Figure 21: Figures 21A-L provide nucleic acid sequences of the present invention. The nucleic acid sequences encoding an E1 or E2 protein according to the present invention may be translated (SEQ ID NO 3 to 13, 21-31, 35 and 41-49 are translated in a reading frame starting from residue number 1, SEQ ID NO 37-39 are translated in a reading frame starting from residue number 2), into the amino

acid sequences of the respective E1 or E2 proteins as shown in the sequence listing.--

Page 38, delete the paragraph spanning lines 31-34, and insert the following therefor:

--Figures 35A-1 to 35A-8: Antibody levels to the different HCV antigens (Core 1, Core 2, E2HCVR, NS3) for NR and LTR followed during treatment and over a period of 6 to 12 months after treatment determined by means of the LIAscan method. The average values are indicated by the curves with the open squares.--

Page 39, delete the paragraph spanning lines 1-4, and insert the following therefor:

--Figures 35B-1 to 35B-8: Antibody levels to the different HCV antigens (NS4, NS5, E1 and E2) for NR and LTR followed during treatment and over a period of 6 to 12 months after treatment determined by means of the LIAscan method. The average values are indicated by the curve with the open squares.--

Page 39, delete the paragraph spanning lines 6 and 7, and insert the following therefor:

--Figures 36A and 36 B: Average E1 antibody (E1Ab) and E2 antibody (E2Ab) levels in the LTR and NR groups.—

Page 39, delete the paragraph spanning lines 8 and 9, and insert the following therefor:

--Figures 37A-D: Averages E1 antibody (E1Ab) levels for non-responders (NR) and long term responders (LTR) for type 1b and type 3a.--

IN THE ABSTRACT

Insert the attached Abstract after the claims pages.

IN THE DRAWINGS

Insert the attached formal drawings for the originally-filed drawings.

IN THE CLAIMS

Cancel claims 1-48, without prejudice.

Add the following claims.

--49. (new) An isolated antibody selected from the group consisting of an E1 specific monoclonal antibody, an E2 specific monoclonal antibody and an E1/E2 specific monoclonal antibody.

50. (new) The monoclonal antibody according to claim 49 which has been produced from a mammal immunized with a composition comprising purified recombinant HCV single or specific oligomeric recombinant envelope proteins selected from the group consisting of at least one of an E1 protein, an E2 protein and an E1/E2 complex.

51. (new) The monoclonal antibody according to claim 50 wherein said recombinant HCV envelope proteins are produced by a recombinant mammalian cell.

52. (new) The monoclonal antibody of claim 51 wherein said mammalian cell is infected with recombinant vaccinia virus carrying DNA for expressing said HCV envelope proteins.

53. (new) The monoclonal antibody according to claim 50 wherein said recombinant HCV envelope proteins are produced by a recombinant yeast cell.

54. (new) The monoclonal antibody according to claim 50 wherein said recombinant HCV envelope proteins are the expression product of at least one of the following recombinant vectors:

a) a recombinant vector comprising a vector sequence, a prokaryotic, eukaryotic or viral promoter sequence followed by a nucleotide sequence allowing the expression of said single or specific oligomeric protein selected from the group consisting of at least one of an E1 protein, E2 protein, and an E1/E2 complex;

b) a recombinant vector according to (a), with said nucleotide sequence being characterized further in that it encodes a single HCV E1 protein starting in the region

between amino acid positions 1 and 192 and ending in the region between amino acid positions 250 and 400;

c) a recombinant vector according to (b), with said nucleotide sequence being characterized further in that it encodes a single HCV E1 protein starting in the region between amino acid positions 117 and 192 and ending in the region between amino acid positions 263 and 400;

d) a recombinant vector according to (b) or (c), with said nucleotide sequence being characterized further in that in encodes a single HCV E1 protein bearing a deletion of the first hydrophobic domain between positions 264 to 293, plus or minus 8 amino acids;

e) a recombinant vector according to (a), with said nucleotide sequence being characterized further in that in encodes a single HCV E2 protein starting in the region between amino acid positions 290 and 406 and ending in the region between amino acid positions 600 and 820;

f) a recombinant vector according to (e), with said nucleotide sequence being characterized further in that it ends at any of amino acid positions 623, 650, 661, 673, 710, 715, 720, 746 or 809;

g) a recombinant vector according to any one of (b)-(f), said nucleotide sequence further comprising a 5'-terminal ATG codon and a 3'-terminal stop codon; and

h) a recombinant vector according to any one of (b)-(g) further comprising a factor Xa cleavage site and/or 3 to 10 histidine codons positioned 3'-terminally to said nucleotide sequence.

55. (new) A monoclonal antibody raised upon immunization with a composition comprising at least one of the following E1 or E2 peptides:

E1-31 (SEQ ID NO:56) spanning amino acids 181 to 200 of the Core/E1 V1 region,

E1-33 (SEQ ID NO:57) spanning amino acids 193 to 212 of the E1 region,

E1-35 (SEQ ID NO:58) spanning amino acids 205 to 224 of the E1 V2 region (epitope B),

E1-35A (SEQ ID NO:59) spanning amino acids 208 to 227 of the E1 V2 region (epitope B),

1bE1 (SEQ ID NO:53) spanning amino acids 192 to 228 of E1 regions V1, C1, and V2 regions (containing epitope B),

E1-51 (SEQ ID NO:66) spanning amino acids 301 to 320 of the E1 region,

E1-53 (SEQ ID NO:67) spanning amino acids 313 to 332 of the E1 C4 region (epitope A),

E1-55 (SEQ ID NO:68) spanning amino acids 325 to 344 of the E1 region,

Env 67 or E2-67 (SEQ ID NO:72) spanning amino acid positions 397 to 418 of the E2 region (epitope A),

Env 69 or E2-69 (SEQ ID NO:73) spanning amino acid positions 409 to 428 of the E2 region (epitope A),

Env 23 or E2-23 (SEQ ID NO:86) spanning positions 583 to 602 of the E2 region (epitope E),

Env 25 or E2-25 (SEQ ID NO:87) spanning positions 595 to 614 of the E2 region (epitope E),

Env 27 or E2-27 (SEQ ID NO:88) spanning positions 607 to 626 of the E2 region (epitope E),

Env 178 or E2-178 (SEQ ID NO:83) spanning positions 547 to 586 of the E2 region (epitope D), and

Env 13B or E2-13B (SEQ ID NO:82) spanning positions 523 to 542 of the E2 region (epitope C).

56. (new) A monoclonal antibody raised upon immunization with a composition comprising at least one of the following E2 conformational epitopes:

epitope F recognized by monoclonal antibodies 15C8C1, 12D11F1, and 8G10D1H9,

epitope G recognized by monoclonal antibody 9G3E6,

epitope H (or C) recognized by monoclonal antibodies 1003C4 and 4H6B2, and

epitope I recognized by monoclonal antibody 17F2C2.

57. (new) Kit for determining the presence of HCV antigens present in a biological sample, comprising:

at least one E1, E2, or E1/E2 specific monoclonal antibody according to claim 49,

a buffer or components necessary for producing the buffer enabling binding reaction between these antibodies and the HCV antigens present in said biological sample,

a means for detecting the immune complexes formed in the preceding binding reaction.

58. (new) An isolated antibody of claim 49 which is an E1 specific monoclonal antibody or an E2 specific monoclonal antibody.

59. (new) The monoclonal antibody according to claim 51 which is an E1 specific monoclonal antibody or an E2 specific monoclonal antibody.

60. (new) The monoclonal antibody according to claim 54 wherein said single or specific oligomeric protein of (a) is selected from the group consisting of an E1 protein and an E2 protein.

61. (new) Kit for determining the presence of HCV antigens present in a biological sample, comprising:

at least one E1 or E2 specific monoclonal antibody according to claim 58,

a buffer or components necessary for producing the buffer enabling binding reaction between these antibodies and the HCV antigens present in said biological sample,

a means for detecting the immune complexes formed in the preceding binding reaction.

62. (new) An isolated antibody selected from the group consisting of an E1 specific monoclonal antibody, an E2 specific monoclonal antibody, and an E1/E2 specific monoclonal antibody, which has been produced from a mammal immunized with a recombinant HCV single or specifically oligomerized envelope viral protein

selected from the group consisting of E1, E2 or E1/E2 viral proteins obtained from a method comprising the steps of:

(a) optionally lysing host cells expressing said viral protein;

(b) optionally recovering said viral protein ;

(c) cleaving disulphide bonds of said viral protein with a disulphide bond cleaving agent to form a cleaved protein;

(d) preventing disulphide bond reformation of said cleaved viral protein with at least one of an SH group blocking agent and low pH condition; and

(e) purifying the cleaved viral protein obtained in step (d) to produce a viral protein which is at least 80% purified.

63. (new) An isolated antibody according to claim 62 wherein said method further comprises desalting said purified viral protein of step (e).

64. (new) An isolated antibody according to claim 62 wherein step (b) or step (e) further comprises a chromatographic recovery.

65. (new) An isolated antibody according to claim 63 wherein step (b) or step (e) further comprises a chromatographic recovery.

66. (new) An isolated antibody according to claim 62 wherein step (b) further comprises an affinity chromatography.

67. (new) An isolated antibody according to claim 62 wherein step (a) further comprises addition of an SH group blocking agent.

68. (new) An isolated antibody according to claim 62 wherein said SH group blocking agent is N-ethylmaleimide.

69. (new) An isolated antibody according to claim 63 wherein said SH group blocking agent is N-ethylmaleimide.

70. (new) An isolated antibody according to claim 64 wherein said affinity chromatography comprises lectin-chromatography or immunoaffinity chromatography with at least one of an anti-E1 specific monoclonal antibody or an anti-E2 specific monoclonal antibody.

71. (new) An isolated antibody according to claim 65 wherein said affinity chromatography comprises lectin-chromatography or immunoaffinity chromatography with at least one of an anti-E1 specific monoclonal antibody or an anti-E2 specific monoclonal antibody.

72. (new) An isolated antibody according to claim 62 wherein said cleaving comprises partial cleaving conditions including addition of a detergent.

73. (new) An isolated antibody according to claim 62 wherein said disulphide bond cleaving agent is dithiothreitol.

74. (new) An isolated antibody according to claim 72 wherein said detergent comprises N-Dodecyl-N,N-dimethylglycine.

75. (new) An isolated antibody according to claim 73 wherein said dithiothreitol is present at a concentration of 0.1 to 50 mM.

76. (new) An isolated antibody according to claim 75 wherein said dithiothreitol is present at a concentration of 0.1 to 20 mM.

77. (new) An isolated antibody according to claim 75 said dithiothreitol is present at a concentration of 0.5 to 10 mM.

78. (new) An isolated antibody according to claim 74 wherein said detergent is present at a concentration of 1 to 10%.

79. (new) An isolated antibody according to claim 74 wherein said detergent is present at a concentration of 3.5%.

80. (new) An isolated antibody according to claim 62 wherein said purified protein is at least 95% pure.

81. (new) An isolated antibody according to claim 63 wherein said purified protein is at least 95% pure.

82. (new) An isolated antibody according to claim 62 wherein said purified protein is at least 97% pure.

83. (new) An isolated antibody according to claim 62 wherein said purified protein is at least 98% pure.

84. (new) An isolated antibody according to claim 62 wherein said purified protein is at least 99% pure.

85. (new) An isolated antibody according to claim 62 wherein said purified protein is at least 90% pure.

86. (new) An isolated antibody according to claim 63 wherein said purified protein is at least 90% pure.

87. (new) An isolated antibody of claim 50 wherein said at least one of an E1 protein, an E2 protein and an E1/E2 complex is at least 80% pure.

88. (new) An isolated antibody of claim 50 which has been produced from a mammal immunized with a composition comprising purified recombinant HCV single or

specific oligomeric recombinant envelope proteins selected from the group consisting of at least one of an E1 protein and an E2 protein.

89. (new) The isolated antibody of claim 50 wherein said mammal is immunized with a composition comprising purified recombinant HCV single or specific oligomeric recombinant envelope proteins selected from the group consisting of at least one of an E1 and an E2 protein and said at least one of an E1 protein and an E2 protein is at least 80% pure.

90. (new) The isolated protein of claim 87 wherein said protein or complex is at least 90% pure.

91. (new) The isolated protein of claim 89 wherein said protein is at least 90% pure.

92. (new) The isolated protein of claim 87 wherein said protein or complex is at least 95% pure.

93. (new) The isolated protein of claim 89 wherein said protein is at least 95% pure.

94. (new) The isolated protein of claim 87 wherein said protein or complex is at least 97% pure.

95. (new) The isolated protein of claim 89 wherein said protein is at least 97% pure.

96. (new) The isolated protein of claim 87 wherein said protein or complex is at least 97% pure.

97. (new) The isolated protein of claim 89 wherein said protein is at least 90% pure.

98. (new) The isolated protein of claim 87 wherein said protein or complex is at least 99% pure.

99. (new) The isolated protein of claim 89 wherein said protein is at least 99% pure.--

REMARKS

The specification and claims have been amended above as provided in the parent application Serial No. 09/928,017. The attached formal drawings are the same as those submitted in the parent application. No new matter has been added. The attached paper copy of the Sequence Listing is the same as that filed in the parent application. A separate Request regarding the computer readable copy of the Sequence Listing is attached.

An early and favorable Action on the merits in the above-identified application is requested.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

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MARKED-UP SPECIFICATION

Page 36, delete the paragraph spanning lines 23-29, and insert the following therefor:

--Figure 21: Figures 21A-L provide nucleic acid sequences of the present invention. The nucleic acid sequences encoding an E1 or E2 protein according to the present invention may be translated (SEQ ID NO 3 to 13, 21-31, 35 and 41-49 are translated in a reading frame starting from residue number 1, SEQ ID NO 37-39 are translated in a reading frame starting from residue number 2), into the amino acid sequences of the respective E1 or E2 proteins as shown in the sequence listing.--

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